

GSK-3: tricks of the trade for a multi-tasking kinase

Bradley W. Doble and James R. Woodgett*

Ontario Cancer Institute, 610 University Avenue, Toronto, Ontario M5G 2M9, Canada

*Author for correspondence (e-mail: jwoodget@uhnres.utoronto.ca)

Journal of Cell Science 116, 1175–1186 © 2003 The Company of Biologists Ltd
doi:10.1242/jcs.00384

Summary

Glycogen synthase kinase 3 (GSK-3) is a multifunctional serine/threonine kinase found in all eukaryotes. The enzyme is a key regulator of numerous signalling pathways, including cellular responses to Wnt, receptor tyrosine kinases and G-protein-coupled receptors and is involved in a wide range of cellular processes, ranging from glycogen metabolism to cell cycle regulation and proliferation. GSK-3 is unusual in that it is normally active in cells and is primarily regulated through inhibition of its activity. Another peculiarity compared with other protein kinases is its preference for primed substrates, that is, substrates previously phosphorylated by another kinase. Several recent advances have improved our understanding of GSK-3 regulation in multiple pathways. These include the solution of the crystal structure of GSK-3, which has

provided insight into GSK-3's penchant for primed substrates and the regulation of GSK-3 by serine phosphorylation, and findings related to the involvement of GSK-3 in the Wnt/β-catenin and Hedgehog pathways. Finally, since increased GSK-3 activity may be linked to pathology in diseases such as Alzheimer's disease and non-insulin-dependent diabetes mellitus, several new GSK-3 inhibitors, such as the aloisines, the paullones and the maleimides, have been developed. Although they are just starting to be characterized in cell culture experiments, these new inhibitors hold promise as therapeutic agents.

Key words: Signal transduction, Phosphorylation, Wnt signalling, Insulin signalling, Protein structure-function

Introduction

Glycogen synthase kinase 3 (GSK-3) is a serine/threonine kinase that was first isolated and purified as an enzyme capable of phosphorylating and inactivating the enzyme glycogen synthase (Embi et al., 1980; Woodgett and Cohen, 1984). We now know that, beyond its role in glycogen metabolism, GSK-3 acts as a downstream regulatory switch that determines the output of numerous signalling pathways initiated by diverse stimuli (reviewed in Frame and Cohen, 2001; Grimes and Jope, 2001; Woodgett, 2001). The pathways in which GSK-3 acts as a key regulator, when dysregulated, have been implicated in the development of human diseases such as diabetes, Alzheimer's disease, bipolar disorder and cancer.

Given its involvement in many pathophysiological processes and diseases, GSK-3 is a tempting therapeutic target. However, its involvement in multiple pathways also raises the issue of selectivity – how might we impact one process while leaving others untouched? We must also assess the full spectrum of GSK-3 functions to avoid later surprises. For example, although inhibition of GSK-3 may be desirable in one context (e.g. in preventing neuronal apoptosis), it could have serious implications for another – for example, it might accelerate hyperplasia by deregulating β-catenin. That said, the recent development of small molecule inhibitors of GSK-3 has provided new tools for visualizing the cell from the perspective of GSK-3. Since it is highly active in most cell types, regulation of substrate phosphorylation occurs either by inactivation of GSK-3 or by changing substrate accessibility or recognition. Here, we present an overview of the various processes in which GSK-3 plays a key role and describe the current status of knowledge of GSK-3 regulation.

GSK-3 variants

There are two mammalian GSK-3 isoforms encoded by distinct genes: GSK-3α and GSK-3β (Woodgett, 1990) (Fig. 1). GSK-3α has a mass of 51 kDa, whereas GSK-3β is a protein of 47 kDa. The difference in size is due to a glycine-rich extension at the N-terminus of GSK-3α. Although highly homologous within their kinase domains (98% identity), the two gene products share only 36% identity in the last 76 C-terminal residues (Woodgett, 1990). Homologues of GSK-3 exist in all eukaryotes examined to date and display a high degree of homology; isoforms from species as distant as flies and humans display >90% sequence similarity within the kinase domain (reviewed in Ali et al., 2001).

GSK-3α and GSK-3β, although structurally similar, are not functionally identical. This became obvious upon ablation of the GSK-3β isoform in mice, which results in an embryonic lethal phenotype (Hoeflich et al., 2000). Embryos carrying homozygous deletions of exon 2 of GSK-3β die around embryonic day 16 owing to massive liver degeneration caused by extensive hepatocyte apoptosis. The inability of GSK-3α to rescue the GSK-3β-null mice indicates that the degenerative liver phenotype arises specifically from the loss of the beta isoform. This phenotype is remarkably similar to that of animals lacking RelA or I-κB kinase 2 (Beg et al., 1995; Li et al., 1999), components of the NF-κB signalling pathway (Ghosh and Karin, 2002; Rothwarf and Karin, 1999). Regulation of NF-κB nuclear import is not disrupted in embryonic fibroblasts isolated from the GSK-3β-null mice, which indicates that GSK-3 affects some other level of NF-κB regulation. The specific target(s) of GSK-3β in the regulation of NF-κB signalling has not been identified. Other protein

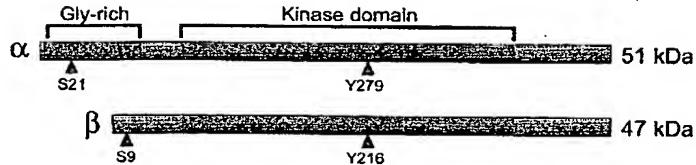


Fig. 1. Schematic representation of mammalian GSK-3 α and GSK-3 β . Sites of serine and tyrosine phosphorylation are indicated with blue arrowheads. The glycine-rich N-terminal domain unique to GSK-3 α and the conserved kinase domain shared by both isoforms are highlighted.

kinases can phosphorylate the p65 subunit of NF- κ B, increasing transactivation (Jang et al., 2001; Wang et al., 2000). GSK-3 β can phosphorylate the C-terminal domain (residues 354–551) of p65 in vitro (Schwabe and Brenner, 2002), but further studies are required to verify if p65 is a physiological GSK-3 β substrate and to assess the effect of this modification. The phenotype of mice lacking GSK-3 α has not yet been reported.

A minor (~15% of total) splice variant of GSK-3 β , GSK-3 β 2, has recently been identified and contains a 13-residue insert within the kinase domain (Mukai et al., 2002). Analysis of the in vitro kinase activity of GSK-3 β 2 revealed reduced activity towards the microtubule-associated protein tau, compared with ‘unspliced’ GSK-3 β . An antibody selective for the novel splice-insertion polypeptide revealed that GSK-3 β 2 is localized primarily to neuronal cell bodies, unlike unspliced GSK-3 β , which is also found in neuronal processes. It is unclear whether these substrate and subcellular localization differences can be generalized to other proteins or cells. Given the location of the insert within a highly conserved sequence, it probably forms a loop/hook, which might allow differential binding of the splice variant to scaffolding proteins that then expose the isoform to a distinct subset of target proteins (Mukai et al., 2002).

GSK-3 phosphorylation: insights from crystal structures

1) T-loop phosphorylation

Three groups recently determined the crystal structure of GSK-3 (Bax et al., 2001; Dajani et al., 2001; ter Haar et al., 2001). The structure has provided insight into both its regulation and its preference for primed, pre-phosphorylated substrates. Protein kinases related to GSK-3, such as CDK2, p38 γ and ERK2, require phosphorylation of residues in their activation loops (T-loops) as a prerequisite for activity (Bellon et al., 1999; Brown et al., 1999; Canagarajah et al., 1997). A phosphothreonine is used by all three related kinases to align key β -strand and α -helical domains. The phosphorylation of a T-loop tyrosine is also required by p38 γ and ERK2 to open up the catalytic site for substrate access. The T-loop of GSK-3 is tyrosine phosphorylated at Y216 and Y279 in GSK-3 β and GSK-3 α , respectively, but not threonine phosphorylated. Y216/Y279 phosphorylation could play a role in forcing open the substrate-binding site, but there appear to be no constraints preventing the open conformation in the unphosphorylated state (Dajani et al., 2001). Thus, T-loop tyrosine phosphorylation of GSK-3 might facilitate substrate phosphorylation but is not strictly required for kinase activity (Dajani et al., 2001).

GSK-3 has an unusual preference for target proteins that are pre-phosphorylated at a ‘priming’ residue located C-terminal

to the site of GSK-3 phosphorylation (Fiol et al., 1987). The consensus sequence for GSK-3 substrates is Ser/Thr-X-X-Ser/Thr-P, where the first Ser or Thr is the target residue, X is any amino acid (but often Pro), and the last Ser-P/Thr-P is the site of priming phosphorylation. Although not strictly required, priming phosphorylation greatly increases the efficiency of substrate phosphorylation of most GSK-3 substrates by 100–1000-fold (Thomas et al., 1999). For example, glycogen synthase, the prototypical primed substrate, requires priming phosphorylation by casein kinase II (CK2) and then undergoes sequential multisite phosphorylation by GSK-3 (Fiol et al., 1988; Fiol et al., 1990).

The function expected for the ‘missing’ phosphothreonine in the GSK-3’s T-loop is believed to be replaced by the phosphorylated residue of a primed substrate, which binds to a positively charged pocket comprising R96, R180 and K205 (residue numbers for GSK-3 β). This not only optimizes the orientation of the kinase domains but also places the substrate at the correct position within the catalytic groove for phosphorylation to occur. There are some substrates that lack a priming site. These proteins often display negatively charged residues at or near the priming position that may mimic a phospho-residue.

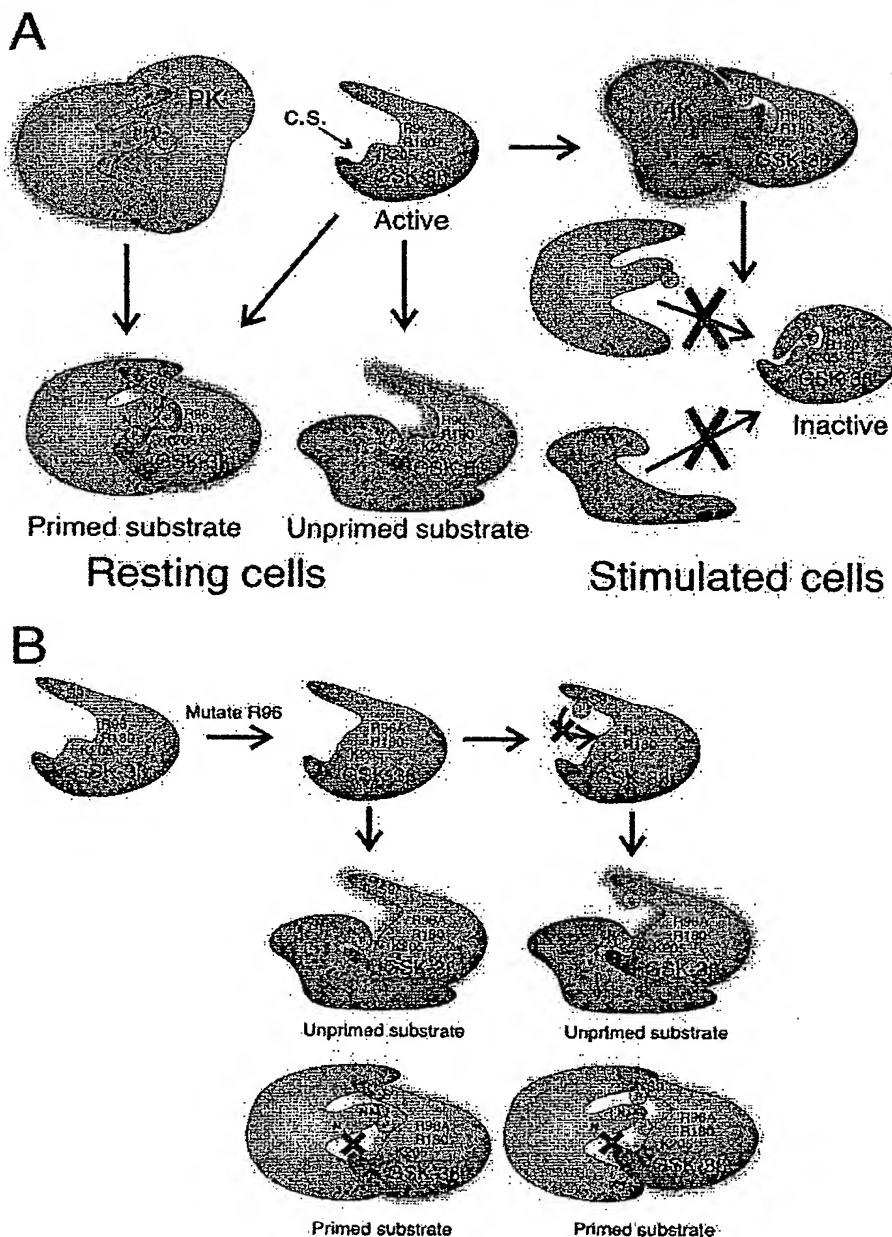
2) Inhibitory serine phosphorylation

Stimulation of cells with insulin causes inactivation of GSK-3 through a phosphoinositide 3-kinase (PI 3-kinase)-dependent mechanism. PI-kinase-induced activation of PKB (also termed Akt) results in PKB phosphorylation of both GSK-3 isoforms (S9 of GSK-3 β ; S21 of GSK-3 α) (Cross et al., 1995), which inhibits GSK-3 activity. This leads to the dephosphorylation of substrates including glycogen synthase and eukaryotic protein synthesis initiation factor-2B (eIF-2B), resulting in their functional activation and consequent increased glycogen and protein synthesis (reviewed in Cohen et al., 1997).

Numerous other stimuli also lead to inactivation of GSK-3 through S9/S21 phosphorylation, including growth factors such as EGF and PDGF that stimulate the GSK-3-inactivating kinase p90^{RSK} (also known as MAPKAP-K1) through MAP kinases (Brady et al., 1998; Saito et al., 1994), activators of p70 ribosomal S6 kinase (p70S6K) such as amino acids, (Armstrong et al., 2001; Krause et al., 2002; Terruzzi et al., 2002), activators of cAMP-activated protein kinase (PKA) (Fang et al., 2000; Li et al., 2000; Tanji et al., 2002) and PKC activators (Ballou et al., 2001; Fang et al., 2002).

The crystal structure of GSK-3 also helps to explain the inhibitory role of this serine phosphorylation (see Fig. 2A). Phosphorylation of S9/S21 creates a primed pseudosubstrate that binds intramolecularly to the positively charged pocket mentioned above. This folding precludes phosphorylation of substrates because the catalytic groove is occupied. Note that

Fig. 2. (A) Regulation of GSK-3 β activity by serine phosphorylation. In the resting cell, GSK-3 β is constitutively active. Both unprimed substrates and substrates phosphorylated by a priming kinase (PK) are capable of being phosphorylated by the active GSK-3 β . The priming phospho-residue at position N + 4, binds a pocket of positive charge arising from the arginine (R) and lysine (K) residues indicated. This directs a serine or threonine at position N to the active catalytic site (C.S.). When an inactivating kinase (IK) such as PKB/Akt phosphorylates GSK-3 β on serine 9 (S9), the phosphorylated N-terminus becomes a primed pseudo-substrate that occupies the positive binding pocket and active site of the enzyme, acting as a competitive inhibitor for true substrates. This prevents phosphorylation of any substrates. (B) Effect of mutating arginine 96 to alanine (R96A) on GSK-3 β activity. Since arginine 96 is a crucial component of the positive pocket that binds primed substrates, its mutation to an uncharged alanine residue disrupts the pocket so that primed substrates can no longer bind. The enzyme retains activity. Also, the S9-phosphorylated pseudosubstrate is no longer capable of inactivating the enzyme. As a consequence, GSK-3 β , whether S9-phosphorylated or not, can phosphorylate unprimed substrates, but not primed substrates. Note that unprimed and primed substrates interact with GSK-3 through different interfaces.



the mechanism of inhibition is competitive. A consequence of this is that primed substrates, in high enough concentrations, out-compete the pseudosubstrate and thus become phosphorylated (Frame et al., 2001). Thus, although less efficient, unprimed substrates may provide a more accurate measure of GSK-3 activity in kinase assays performed in vitro. Mutation of Arg96 to alanine disrupts the pocket of positive charge (Fig. 2B) (Frame et al., 2001). Interestingly, although

peptide of 11 residues that matches the phosphorylated N-terminus of GSK-3 β peptide-11 competitively inhibits both primed and unprimed substrates, a truncated version of this peptide (NTptide-8) inhibits phosphorylation only of primed substrates (Frame et al., 2001). Thus, small molecule inhibitors modeled to fit in the positively charged pocket of the GSK-3 kinase domain could potentially be very effective for selective inhibition of primed substrates.

Physiological roles for tyrosine phosphorylation

The physiological significance of phosphorylation of Y216 of GSK-3 β in mammalian cells is unclear, since this phosphorylation is constitutive in resting cells (Hughes et al., 1993). The kinase(s) responsible has not been identified, although the tyrosine kinase (Zak1) that phosphorylates the Y216 equivalent is known in the slime mould (Kim et al., 1999). Mammalian GSK-3 β expressed in bacteria shows evidence of autophosphorylation on tyrosine, serine and threonine residues, which raises the possibility that tyrosine phosphorylation of GSK-3 in mammalian cells is an autocatalytic event (Wang et al., 1994). GSK-3 immunoprecipitated from mammalian cells does not show this behaviour, however (Hughes et al., 1993).

Although GSK-3 is tyrosine phosphorylated in resting cells, the level may not be stoichiometric. Apoptotic stimuli such as staurosporine treatment or neurotrophic factor withdrawal increase GSK-3 activity and tyrosine phosphorylation in certain neuronal cell lines (Bhat et al., 2000; Bijur and Jope, 2001). Moreover, LPA, through a pathway involving the G proteins $G_{\alpha 12}$ and $G_{\alpha 13}$, also increases GSK-3 activity in neuronal cells at least in part through enhanced tyrosine phosphorylation of Y216/Y279 (Sayas et al., 2002). Since treatment of primary neurons with LPA results in neurite retraction, treatment of a neuroblastoma cell line, Neuro2A, results in cell rounding (Sayas et al., 2002), and LPA also causes apoptosis of adult neurons (Steiner et al., 2000), this provides further circumstantial evidence for a role for tyrosine phosphorylation in apoptosis.

Recent work has revealed two possible candidates for kinases that might tyrosine phosphorylate GSK-3 under these circumstances. Transient increases in intracellular calcium increase GSK-3-mediated phosphorylation of the microtubule-associated protein tau, and the increased GSK-3 activity is attributed to elevated GSK-3 tyrosine phosphorylation (Hartigan and Johnson, 1999). The tyrosine kinase that may be responsible for the phosphorylation of GSK-3 is proline-rich tyrosine kinase 2 (PYK2)[†], a calcium-sensitive enzyme. Active, but not kinase-dead, PYK2 increases tyrosine phosphorylation of GSK-3 both in PYK2-transfected cells and *in vitro*, and PYK2 and GSK-3 β co-immunoprecipitate in cells transfected with PYK2 (Hartigan et al., 2001). The Fyn tyrosine kinase is another potential player: insulin treatment of SH-SY5Y cells for 1 minute causes an increase in association of Fyn with GSK-3 β , a transient increase in GSK-3 β activity with a concomitant transient increase in GSK-3 β phosphorylation on Y216 and a transient increase in tau phosphorylation. The short duration of insulin treatment is crucial for the observed increase in GSK-3 activity (Lesort et al., 1999). Prolonged treatment with insulin, besides the well characterized inhibition of GSK-3 through serine phosphorylation (discussed above), has also been reported to inhibit GSK-3 through tyrosine dephosphorylation in Chinese hamster ovary cells (Murai et al., 1996). Further characterization of the proposed GSK-3-phosphorylating tyrosine kinases is required to substantiate a role for them in physiological processes.

The role of tyrosine phosphorylation of GSK-3 in

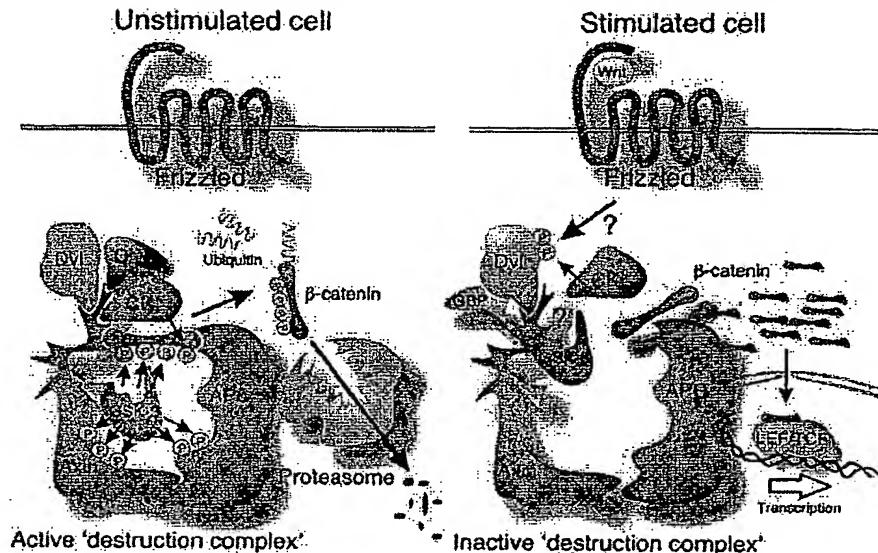
Dictyostelium is better understood. Zak1 is essential for activation of GSK-3 during developmental patterning and is regulated downstream of certain cyclic AMP receptors involved in chemotaxis (Kim et al., 1999; Plyte et al., 1999). Unfortunately, identification of a mammalian orthologue of Zak1 has so far proven elusive. Kim et al. have recently described an opposing role for a protein tyrosine phosphatase, which dephosphorylates GSK-3 on tyrosine residues and results in a prestalk fate (Kim et al., 2002). Interestingly, phosphorylation of two tyrosine residues, both residing in the activation loop of GSK-3, appears to be modulated by cAMP in *Dictyostelium* (Kim et al., 2002). Both of these tyrosine residues, Y214 and Y220, are conserved in mammals (Y216 and Y219, respectively). Thus, Y219 – in addition to Y216 – might therefore play a role in GSK-3 regulation in mammals.

The role of GSK-3 in the Wnt/ β -catenin pathway

The Wnts are a family of secreted, cysteine-rich, glycosylated, protein ligands that influence cell growth, differentiation, migration and fate (Miller, 2002; Polakis, 2000; Smalley and Dale, 1999). Members of the Wnt family have been identified in organisms ranging in complexity from the cnidarian Hydra to humans; at least 19 different Wnts exist in mammals (Miller, 2002). Severe developmental defects usually occur in mice that have defective Wnt signalling. One of the pathways regulated by Wnt molecules is termed the canonical Wnt pathway or the Wnt/ β -catenin pathway (reviewed in Huelsken and Behrens, 2002; Polakis, 2000; Seidensticker and Behrens, 2000; Sharpe et al., 2001) (Fig. 3). Here, we limit discussion of Wnt signalling to this signalling pathway. Wnt signal transduction ultimately results in the activation of genes regulated by the T-cell factor (TCF)/lymphoid enhancer factor (LEF) family of architectural transcription factors (reviewed in Barker et al., 2000; Brantjes et al., 2002; Novak and Dedhar, 1999). The effector molecule responsible for activating TCF/LEF-responsive genes is β -catenin, which serves as a transactivator that binds to DNA-bound TCF/LEFs. As in insulin signalling, GSK-3 plays a key inhibitory role in the Wnt pathway. In unstimulated cells, GSK-3 phosphorylates the N-terminal domain of β -catenin, thereby targeting it for ubiquitylation and proteasomal degradation. Exposure of cells to Wnts leads to inactivation of GSK-3 through an as yet unclear mechanism. The phosphoprotein Dishevelled is required, after receptor-ligand interaction, to transduce the signal that results in the inactivation of GSK-3. As a result, β -catenin is dephosphorylated (van Noort et al., 2002) and escapes the ubiquitylation-dependent destruction machinery. Unphosphorylated β -catenin accumulates in the cytoplasm and translocates to the nucleus, where it can associate with the TCF/LEFs and become a transcriptional transactivator. Mutations in β -catenin that prevent its phosphorylation by GSK-3 (gain-of-function mutations) have been found in cancers of the skin, colon, prostate, liver, endometrium and ovary (reviewed in Polakis, 2000). Many of the components of the Wnt pathway, and their functional roles, were first identified in studies of the Wnt pathway in *Drosophila melanogaster*, which is driven by the Wnt homologue, Wingless (Dierick and Bejsovec, 1999; Manoukian and Woodgett, 2002; Siegfried and Perrimon, 1994). In *Drosophila*, the GSK-3 homologue is termed Shaggy (Bourouis et al., 1990) or Zeste-white3 (Siegfried et al., 1990). Phosphorylation of β -catenin by GSK-3 occurs in a complex

[†]Also known as cell adhesion kinase beta; focal adhesion kinase 2; protein tyrosine kinase 2 beta; cell adhesion kinase beta; calcium-dependent kinase; realted adhesion focal tyrosine kinase.

Fig. 3. Central role of GSK-3 in the Wnt/β-catenin pathway. In unstimulated cells, CKI phosphorylates β-catenin on S45, priming it for subsequent phosphorylation by GSK-3 (S41, S37, S33), which targets β-catenin for ubiquitylation and proteasomal degradation. The ankyrin repeat protein, Diversin (Div), may help recruit CKI to the destruction complex. Wnt stimulation activates the receptor Frizzled, which then signals through Dishevelled (Dvl), using an unclear mechanism, to inactivate β-catenin phosphorylation. Unphosphorylated β-catenin accumulates and then translocates to the nucleus where it transactivates genes regulated by TCF/LEF transcription factors. The GSK-3-binding protein (GBP/FRAT) may be involved in transmission of a Wnt signal by regulating binding of GSK-3 to the scaffold protein, axin.



sometimes referred to as the destruction complex, which consists minimally of the proteins GSK-3, β-catenin, axin/conductin and adenomatous polyposis coli (APC) (Hinoi et al., 2000). APC is a tumour suppressor protein commonly deleted in familial adenomatous polyposis and sporadic colorectal cancer (Polakis, 1997). Axin (and a related protein known as conductin or axil) harbors several protein-protein interaction domains and serves as a scaffolding protein that holds together the elements of the β-catenin destruction complex. Both Axin and APC are phosphorylated by GSK-3. Phosphorylation of axin by GSK-3 increases its stability and binding to β-catenin (Ikeda et al., 1998; Jho et al., 1999; Yamamoto et al., 1999). Phosphorylation of APC increases its binding to β-catenin (Rubinfeld et al., 1996).

Five groups have recently determined that β-catenin is also a primed substrate for GSK-3, with casein kinase I (CKI) acting as the priming kinase (Amit et al., 2002; Hagen et al., 2002; Hagen and Vidal-Puig, 2002; Liu et al., 2002; Sakanaka, 2002; Yanagawa et al., 2002). CKI phosphorylates S45, which lies four residues C-terminal to three GSK-3 targets at serines S33, S37 and S41. As a priming kinase, CKI functions as a negative regulator of Wnt signalling since it promotes GSK-3 function. Such a role contrasts with that proposed in several previous reports, which identified CKI as a positive transducer of Wnt signalling (Gao et al., 2002; Kishida et al., 2001; Lee et al., 2001; McKay et al., 2001). CKI binds to axin and Dishevelled and phosphorylates not only β-catenin but also axin, Dishevelled and APC. Polakis has proposed a model in which CKI can act as both a positive and a negative regulator of Wnt signalling (Polakis, 2002). In this model, CKI plays a role in the destruction complex as the priming kinase for GSK-3 and is required for transmission of the Wnt signal by assisting in the activation of Dishevelled, perhaps by increasing its affinity for signalling intermediates (see below). A novel ankyrin-repeat-containing protein, Diversin, has been reported to recruit CKI to the destruction complex (Schwarz-Romond et al., 2002).

These data raise the possibility that GSK-3 plays only a latent role in regulation of β-catenin. For example, if CKI activity is directly regulated by Wnt signalling, then phosphorylation of S45 would act as the trigger for subsequent phosphorylation by GSK-3. In this scenario, the activity of GSK-3 could be totally independent of Wnt regulation. However, phosphorylation of S45 appears to be constitutive, at least in some cell types. Although S45 phosphorylation has been reported to decrease upon Wnt stimulation, the phospho-specific antibodies used in those studies also detect S41, one of the GSK-3 targets (Amit et al., 2002). Antibodies selective for phosphoserine 45 do not reveal changes in stoichiometry in response to Wnt (Liu et al., 2002). Clearly, cells have evolved complex mechanisms to titrate β-catenin levels, presumably to allow multiple layers of control.

Another interesting player in the regulation of the Wnt pathway, at least in vertebrates, is a GSK-3-binding protein termed GBP (also known as FRAT[†]) (Farr et al., 2000; Ferkey and Kimelman, 2002; Fraser et al., 2002; Sumoy et al., 1999; Yost et al., 1998). Binding of GBP to GSK-3 precludes GSK-3 from binding to axin and thus interferes with β-catenin phosphorylation. A small peptide derived from FRAT called FRAT-tide is sufficient to prevent axin-GSK-3 interaction and prevents phosphorylation of both axin and β-catenin (Thomas et al., 1999). Comparison of mutations that affect binding of GSK-3 to axin and GBP, as well as analysis of the crystal structure of a GSK-3-FRAT-tide complex, indicates that the binding sites on GSK-3 for GBP/FRAT and axin overlap (Bax et al., 2001; Ferkey and Kimelman, 2002; Fraser et al., 2002). Treatment of *Xenopus* embryo extracts with CKIε increases binding of GBP to Dishevelled (Lee et al., 2001).

GBP also plays a role in the nuclear export of GSK-3 (Franca-Koh et al., 2002). A mutant of GSK-3 that cannot bind to GBP accumulates in the nucleus. Moreover, a peptide that

[†]Frequently rearranged in advanced T-cell lymphocytes

interferes with GBP binding to GSK-3 causes endogenous GSK-3 to accumulate in the nucleus. These findings suggest that GBP regulates access of GSK-3 to substrates partitioned between nuclear and cytoplasmic compartments. Since there are two known mammalian GBP homologues (Freeman et al., 2002), each with dynamically regulated expression patterns during development, GBP could play an important role in modulating GSK-3 function, especially during development. Rather surprisingly, GBP homologues have not been identified in *Drosophila* or *C. elegans*, which indicates that the protein is not a core component of canonical Wnt signalling.

A critical aspect of GSK function in the Wnt pathway is that GSK-3 appears to be insulated from regulators of GSK-3 that lie outside of the Wnt pathway. For example, insulin signalling leads to inhibition of GSK-3 via serine S9/S21 phosphorylation but does not cause accumulation of β -catenin. Conversely, Wnt signalling does not affect insulin signalling (Ding et al., 2000; Yuan et al., 1999). How this insulation occurs is unclear, but it probably stems from the effective sequestration of a fraction of GSK-3 with axin in the destruction complex. Note that tissues from mice lacking GSK-3 β do not show evidence of accumulated β -catenin even though total GSK-3 levels are reduced by 50% and there is zero cellular GSK-3 β . Immunoprecipitation of axin from these tissues reveals that GSK-3 β is simply replaced by GSK-3 α (in wild-type cells, both GSK-3 α and GSK-3 β are found bound to axin) (E. Rubie and J.R.W., unpublished). Since cellular levels of GSK-3 exceed those of axin, the destruction machinery compensates for the loss of GSK-3 β by substituting GSK-3 α .

GSK-3 and the Hedgehog pathway

Groups examining Hedgehog (Hh) signalling in *Drosophila* recently discovered yet another novel function for GSK-3 (Jia et al., 2002; Price and Kalderon, 2002). The Hh pathway is somewhat similar to the Wnt pathway: both Wnt and Hh are secreted signalling proteins involved in embryonic patterning that often work in concert (reviewed in Ingham and McMahon, 2001). Whereas the Wnt pathway uses β -catenin to transduce its signals to the nucleus, the Hh pathway employs a protein called Cubitus interruptus (Ci) in flies or Gli in mammals (Ingham and McMahon, 2001). In the absence of a Hh signal, Ci is targeted for proteolysis. Unlike in Wnt signalling, proteolysis does not result in total degradation of Ci but instead processes it from a 155-residue form (Ci155) to a truncated 75-residue amino acid form (Ci75) that functions as a transcriptional repressor (Aza-Blanc et al., 1997). GSK-3, in combination with CKI and the priming kinase PKA, phosphorylates Ci155 to target it for proteolytic processing in the absence of a Hh signal (Jia et al., 2002; Price and Kalderon, 2002). Activation of Hh signalling results in translocation of full-length Ci155 to the nucleus, where it activates Hh target genes. As in the Wnt pathway, several of the molecules involved in Hh signal transduction have been implicated in cancer (reviewed in Taipale and Beachy, 2001). Notably, familial germline mutations in one allele of the Hh receptor, Patched, are associated with an almost complete penetrance of basal cell carcinoma (Johnson et al., 1996; Taipale, 2001). Although GSK-3 phosphorylation of the mammalian homologues of Ci has yet to be reported, all contain multiple GSK-3 consensus sites next to PKA sites (Ruis i Altaba, 1999;

Jia et al., 2002). It seems likely, on the basis of the conservation of the Wnt pathway, that the Gli proteins will soon be confirmed as GSK-3 substrates.

Other GSK-3 substrates

Numerous putative GSK-3 substrates have roles in a wide spectrum of cellular processes, including glycogen metabolism, transcription, translation, cytoskeletal regulation, intracellular vesicular transport, cell cycle progression, circadian rhythm regulation and apoptosis. Phosphorylation of these substrates by GSK-3 is most often inhibitory, as in the cases of glycogen synthase, β -catenin and Ci. Although a detailed description of the function of all of the substrates is beyond the scope of this review, a list of many of the best-characterized substrates as well as some newly identified substrates, with a brief description of their function and the role of GSK-3 phosphorylation, is shown in Table 1.

Other GSK-3-binding proteins

Besides GBP, axin and APC, several other new GSK-3-binding partners have recently been identified. The tumour-suppressor protein p53, for example, has recently been shown to bind and activate GSK-3 in the nucleus of neuroblastoma cells treated with camptothecin, a topoisomerase I inhibitor that causes DNA damage and subsequently elicits a p53 response (Watcharasit et al., 2002). The activation of GSK-3 occurs without changes in its phosphorylation and is restricted to nuclear GSK-3 and involves an unknown mechanism. In addition, a yeast two-hybrid screen identified the A-kinase-anchoring protein AKAP220 as a GSK-3-binding partner (Tanji et al., 2002). AKAPs are scaffolding proteins, and the interaction between AKAP220 and GSK-3 occurs in a complex that also contains PKA and type I protein phosphatase. The interaction between AKAP220 and GSK-3 enhances PKA-mediated inhibition of GSK-3.

Presenilin 1 (PS1) is one of the two mammalian presenilins identified through association of mutations in these proteins with early-onset familial Alzheimer's disease. Besides having a role in proteolytic processing of proteins at the cell membrane such as amyloid precursor protein, presenilin also binds to β -catenin and has been implicated in regulating its cellular levels. PS1 may also function as a scaffolding protein that binds PKA, GSK-3 and β -catenin (Palacino et al., 2001). This novel quaternary complex functions in a similar manner to the axin complex, using PS1 to couple the priming phosphorylation by PKA (on S45 of β -catenin) to subsequent phosphorylation by GSK-3.

GSK-3 and human disease

Many of the pathways that use GSK-3 as a regulator have links to human diseases. As described above, GSK-3 is a core component of two pathways involved in cell fate determination and morphology, the Wnt and Hedgehog pathways, which are both involved in several forms of human cancer. There are also numerous studies linking GSK-3 to Alzheimer's disease, on the basis of GSK-3-mediated hyperphosphorylation of tau, which is associated with neurofibrillary tangles, one of the hallmarks of Alzheimer's disease (reviewed in De Ferrari and Inestrosa, 2000;

Table 1. Putative GSK-3 substrates

Putative substrate	Function	Effect of phosphorylation by GSK-3	References
Kinesin light chain	Regulatory component of motor protein involved in vesicular transport	Inhibits anterograde vesicular movement	Morfini et al., 2002
Presenilin 1 (PS1)	Transmembrane protein linked to Alzheimer's disease; also binds β -catenin	Increases degradation of C-terminal PS1 fragment	Kirschenbaum et al., 2001
Tau	Microtubule-associated protein; stabilizes microtubules	Reduced microtubule binding; decreased microtubule stability	Hanger et al., 1992
β -catenin	Transcription transactivator	Targets for degradation	Yost et al., 1996
Axin	Scaffold protein in Wnt Pathway	Increased affinity for β -catenin and increased axin stability	Ikeda et al., 1998; Jho et al., 1999; Yamamoto et al., 1999
Adenomatous Polyposis Coli	Wnt pathway component	Increased β -catenin binding; decreased microtubule binding	Rubinfeld et al., 1996
Timeless	Transcription factor regulating <i>Drosophila</i> circadian rhythm	Increased heterodimerization with clock gene called period or increased nuclear transport suggested	Martinek et al., 2001
Nuclear factor of activated T-cells c	Transcription factor; early immune response genes	Decreased DNA binding; increased nuclear export	Beals et al., 1997
Heat shock factor-I	Transcription factor; regulates genes in response to potentially lethal stressors	Inactivates transcription factor activity	Chu et al., 1996
c-Jun	Transcription factor; component of activator protein-1 (AP-1) that regulates many diverse genes	Decreased DNA binding and transactivation	Boyle et al., 1991
c-Myc	Transcription factor; regulates genes involved in cell growth, differentiation and apoptosis	Targets for degradation	Pulverer et al., 1994; Sears et al., 2000
cAMP response element binding protein	Transcription factor; regulates cAMP-responsive genes	Increased transcription factor activity	Fiol et al., 1994
Microphthalmia-associated transcription factor	Transcription factor; regulates tyrosinase expression	Increased binding to tyrosinase promoter	Khaled et al., 2002; Takeda et al., 2000
Cyclin D1	Transcription factor; cell cycle regulation	Increased nuclear export; targets for degradation	Diehl et al., 1998
eIF-2B translation factor	Critical for translation initiation	Inhibits activity	Singh et al., 1996; Welsh and Proud, 1993
Inhibitor-2 Glycogen synthase	Regulatory subunit of phosphatase Glycogen metabolism	Activates phosphatase Inhibits enzyme activity	Park et al., 1994 Dent et al., 1989; Dent et al., 1990; Fiol et al., 1988
Insulin receptor substrate 1	Insulin signalling	Inhibits insulin receptor signalling	Eldar-Finkelman and Krebs, 1997
Acetyl CoA carboxylase	Key lipogenic enzyme	Inactivates enzyme	Hughes et al., 1992
ATP-citrate lyase	Fatty acid synthesis	Inactivates enzyme	Benjamin et al., 1994; Hughes et al., 1992
Mucin 1 / DF3 antigen	Transmembrane glycoprotein that binds β -catenin; overexpressed in human carcinomas	Decreases interaction with β -catenin	Li et al., 1998

Substrates involved in Alzheimer's disease are shaded blue, components of the Wnt pathway are shaded yellow, transcription factors are shaded green and proteins involved in protein, insulin and lipid metabolism are shaded red.

Maccioni et al., 2001; Mattson, 2001). The GSK-3 inhibitor lithium (Stambolic et al., 1996), used for decades as a therapy for bipolar disorder, also implicates GSK-3 activity in the underlying causes of this disease (Detera-Wadleigh, 2001; Gould and Manji, 2002; Manji et al., 1999). In support of a role for GSK-3 in mood regulation is the fact that another mood-stabilizing drug, valproate, also inhibits GSK-3, although probably through an indirect mechanism (Chen et al., 1999; De Samo et al., 2002). Note that lithium and valproate also affect neuronal inositol metabolism, and their mood-stabilizing properties might be related to this effect (Williams, 2002; Hall et al., 2002; Li et al., 2002). Apart from a role in neurological pathologies, GSK-3 may also play a role in the development of non-insulin-dependent diabetes mellitus (NIDDM), a disease often associated with chronic inhibition of muscle glycogen synthase (reviewed in Kaidanovich and Eldar-Finkelman, 2002).

Small molecule inhibitors of GSK-3

Given the association of abnormal GSK-3 activity and various human diseases, GSK-3 is emerging as a potential therapeutic

target, particularly true where its over-expression may be linked to pathology – for example, in NIDDM and neurodegenerative diseases (Eldar-Finkelman, 2002) – but it may also be relevant for some of the more recently identified functions of GSK-3, such as its role in activating NF- κ B, a well characterized target for anti-inflammatory agents (Hoeflich et al., 2000). The best-characterized inhibitor of GSK-3 is lithium (Klein and Melton, 1996; Stambolic et al., 1996). Although fairly specific for GSK-3, compared with other protein kinases, lithium also affects other enzymes, and a relatively high dose is required (K_i is mM) to inhibit GSK-3 activity in cell culture (Stambolic et al., 1996). The mode of inhibition is through competition for Mg^{2+} (Ryves and Harwood, 2001). Recently, the bivalent form of zinc, which mimics insulin action, has also been shown to inhibit GSK-3 when used at a concentration of 15 μ M in a cell culture system (Ilouz et al., 2002). In vitro, another metal ion, beryllium, inhibits GSK-3 to half maximal activity at a concentration of 6 μ M (Ryves et al., 2002).

Several new GSK-3 inhibitors have recently been developed, most of which are ATP competitive (Martinez et al., 2002a; Martinez et al., 2002b). A list of these drugs, their mode of

Table 2. GSK-3 inhibitors

Drug type	Specific drug examples	Mode of action	Clinical effects/effects in cell culture	Reference
Aloisines	Aloisine A, Aloisine B	ATP-competitive	Inhibits cell proliferation	Mettey et al., 2003
Beryllium	N/A	Competes for Mg ²⁺ and ATP	No data	Ryves et al., 2002
Bivalent zinc	N/A	Undetermined; does not compete for substrate	Insulin-mimetic	Houz et al., 2002
Hymenialdisine	Dibromo-hymenialdisine	ATP-competitive	Suppresses inflammation; inhibits tau phosphorylation	Breton and Chabot-Fletcher, 1997; McJee et al., 2000
Indirubins	5,5'-dibromo-indirubin	ATP-competitive	Anti-mitotic; anti-tumoural; inhibits tau phosphorylation	Damiens et al., 2001; Leclerc et al., 2001
Lithium	N/A	Competes for Mg ²⁺	Mood stabilization; prevents polyglutamine toxicity in Huntington's disease	Carmichael et al., 2002; Klein and Melton, 1996; Ryves and Harwood, 2001; Stambolic et al., 1996
Maleimides	Ro 31-8220, SB-216763, SB-415286	ATP-competitive	Insulin-mimetic; prevention of death in culture neuronal	Coghlan et al., 2000; Cross et al., 2001; Hers et al., 1999; Lochhead et al., 2001; Smith et al., 2001
Muscarinic agonists	AF102B, AF150	Unclear	Inhibits tau phosphorylation	Forlenza et al., 2000
N/A, non-applicable.				

action and some of their reported effects in cell culture and patients is shown in Table 2.

There may be a skeleton in the closet, however, for anti-GSK-3 therapeutics. In addition to the problem of its broad range of functions, inhibition of the enzyme could presumably lead to enhanced accumulation of β -catenin, a known oncogene. To mitigate this complication, drugs that selectively target non-axin-associated GSK-3 would be desirable, especially for chronic diseases, such as diabetes. On the positive side, long-term lithium treatment is not associated with enhanced incidence of cancer, although the lithium-treated patient cohort is not typical of the general population.

Perspectives

An enzyme that has as many proposed substrates as GSK-3 requires numerous levels of regulation to confer signal-dependent specificity. Several different mechanisms regulating the phosphorylation of GSK-3 substrates have been described: (1) inactivation of GSK-3 through serine phosphorylation; (2) activation of GSK-3 through tyrosine phosphorylation; (3) inactivation of GSK-3 through tyrosine dephosphorylation; (4) covalent modification of substrates through priming phosphorylation; (5) inhibition or facilitation of GSK-3-mediated substrate phosphorylation through interaction of GSK-3 with binding or scaffolding proteins; (6) targeting of GSK-3 to different subcellular localizations; (7) differential usage of isoforms or splice variants to alter subcellular localization or substrate specificity; and (8) integration of parallel signals conveyed by a single stimulus.

Although our understanding of GSK-3 regulation is increasing, there are still several outstanding issues: (1) putative GSK-3 substrates need to be verified and classified as to whether they require priming phosphorylation; (2) proteins that have been identified as GSK-3-interacting proteins require characterization to identify sites of interaction and mechanisms that regulate binding; (3) the mechanism of Wnt-mediated inactivation of GSK-3 remains to be established; (4) the basis for the specific requirement for GSK-3 β in regulating NF κ B function remains unclear (GSK-3 α does not substitute); and (5) the specificity of newly emerging GSK-3 inhibitors must be validated.

GSK-3 has reliably and regularly provided surprises ever

since its discovery, and several of these have proven to be new signalling paradigms. There is little reason to doubt that there are more revelations in store, but with enhanced tools, such as selective inhibitors, knockout mice and modified binding proteins, there is hope that the true cellular roles for this multi-talented kinase will be appreciated and its utility as a therapeutic target be realized. But there are certainly easier molecules to study...

J.R.W. is a Canadian Institutes of Health Research (CIHR) Senior Scientist and International Scholar of the Howard Hughes Medical Institute and is supported by grants from the Canadian Institutes of Health Research, the Terry Fox Foundation and the National Cancer Institute of Canada. B.W.D. is supported by a CIHR post-doctoral fellowship.

References

- All, A., Hoeftlich, K. P. and Woodgett, J. R. (2001). Glycogen synthase kinase-3: properties, functions, and regulation. *Chem. Rev.* **101**, 2527-2540.
- Amit, S., Hatzubai, A., Birman, Y., Andersen, J. S., Ben-Shushan, E., Mann, M., Ben-Neriah, Y. and Alkalay, I. (2002). Axin-mediated CKI phosphorylation of beta-catenin at Ser 45: a molecular switch for the Wnt pathway. *Genes Dev.* **16**, 1066-1076.
- Armstrong, J. L., Bonavaud, S. M., Tooze, B. J. and Yeaman, S. J. (2001). Regulation of glycogen synthesis by amino acids in cultured human muscle cells. *J. Biol. Chem.* **276**, 952-956.
- Aza-Blanc, P., Ramirez-Weber, F. A., Laget, M. P., Schwartz, C. and Kornberg, T. B. (1997). Proteolysis that is inhibited by hedgehog targets Cubitus interruptus protein to the nucleus and converts it to a repressor. *Cell* **89**, 1043-1053.
- Ballou, L. M., Tian, P. Y., Lin, H. Y., Jiang, Y. P. and Lin, R. Z. (2001). Dual regulation of glycogen synthase kinase-3beta by the alpha1A-adrenergic receptor. *J. Biol. Chem.* **276**, 40910-40916.
- Barker, N., Morin, P. J. and Clevers, H. (2000). The Yin-Yang of TCF/beta-catenin signaling. *Adv. Cancer Res.* **77**, 1-24.
- Bax, B., Carter, P. S., Lewis, C., Guy, A. R., Bridges, A., Tanner, R., Pettman, G., Mannix, C., Culbert, A. A., Brown, M. J. et al. (2001). The structure of phosphorylated GSK-3beta complexed with a peptide, FRATide, that inhibits beta-catenin phosphorylation. *Structure (Camb)* **9**, 1143-1152.
- Beals, C. R., Sheridan, C. M., Turck, C. W., Gardner, P. and Crabtree, G. R. (1997). Nuclear export of NF-ATc enhanced by glycogen synthase kinase-3. *Science* **275**, 1930-1934.
- Beg, A. A., Sha, W. C., Bronson, R. T., Ghosh, S. and Baltimore, D. (1995). Embryonic lethality and liver degeneration in mice lacking the RelA component of NF-kappa B. *Nature* **376**, 167-170.
- Bellon, S., Fitzgibbon, M. J., Fox, T., Hsiao, H. M. and Wilson, K. P. (1999).

- The structure of phosphorylated p38gamma is monomeric and reveals a conserved activation-loop conformation. *Structure Fold Des.* 7, 1057-1065.
- Benjamin, W. B., Pentyala, S. N., Woodgett, J. R., Hod, Y. and Marshak, D. (1994). ATP citrate-lyase and glycogen synthase kinase-3 beta in 3T3-L1 cells during differentiation into adipocytes. *Biochem J.* 300, 477-482.
- Bhat, R. V., Shanley, J., Correll, M. P., Fieles, W. E., Keith, R. A., Scott, C. W. and Lee, C. M. (2000). Regulation and localization of tyrosine-216 phosphorylation of glycogen synthase kinase-3beta in cellular and animal models of neuronal degeneration. *Proc Natl Acad Sci USA* 97, 11074-11079.
- Bijur, G. N. and Jope, R. S. (2001). Proapoptotic stimuli induce nuclear accumulation of glycogen synthase kinase-3 beta. *J Biol Chem.* 276, 37436-37442.
- Bouroults, M., Moore, P., Ruel, L., Grau, Y., Heitzler, P. and Simpson, P. (1990). An early embryonic product of the gene shaggy encodes a serine/threonine protein kinase related to the CDC28/cdc2+ subfamily. *EMBO J.* 9, 2877-2884.
- Boyle, W. J., Smeal, T., Defize, L. H., Angel, P., Woodgett, J. R., Karin, M. and Hunter, T. (1991). Activation of protein kinase C decreases phosphorylation of c-Jun at sites that negatively regulate its DNA-binding activity. *Cell* 64, 573-584.
- Brady, M. J., Bourbonais, F. J. and Saltiel, A. R. (1998). The activation of glycogen synthase by insulin switches from kinase inhibition to phosphatase activation during adipogenesis in 3T3-L1 cells. *J Biol Chem.* 273, 14063-14066.
- Brantjes, H., Barker, N., van Es, J. and Clevers, H. (2002). TCF: Lady Justice casting the final verdict on the outcome of Wnt signalling. *Biol Chem.* 383, 255-261.
- Breton, J. J. and Chabot-Fletcher, M. C. (1997). The natural product hymenialdisine inhibits interleukin-8 production in U937 cells by inhibition of nuclear factor-kappaB. *J Pharmacol Exp Ther.* 282, 459-466.
- Brown, N. R., Noble, M. E., Endicott, J. A. and Johnson, L. N. (1999). The structural basis for specificity of substrate and recruitment peptides for cyclin-dependent kinases. *Nat Cell Biol.* 1, 438-443.
- Canagarajah, B. J., Khokhlovchev, A., Cobb, M. H. and Goldsmith, E. J. (1997). Activation mechanism of the MAP kinase ERK2 by dual phosphorylation. *Cell* 90, 859-869.
- Carmichael, J., Sugars, K. L., Bao, Y. and Rubinstein, D. C. (2002). GSK-3beta inhibitors prevent cellular polyglutamine toxicity caused by the Huntington's disease mutation. *J Biol Chem.* 277, 33791-33798.
- Chen, G., Huang, L. D., Jiang, Y. M. and Manji, H. K. (1999). The mood-stabilizing agent valproate inhibits the activity of glycogen synthase kinase-3. *J Neurochem.* 72, 1327-1320.
- Chu, B., Soncin, F., Price, B. D., Stevenson, M. A. and Calderwood, S. K. (1996). Sequential phosphorylation by mitogen-activated protein kinase and glycogen synthase kinase 3 represses transcriptional activation by heat shock factor-1. *J Biol Chem.* 271, 30847-30857.
- Coghlan, M. P., Culbert, A. A., Cross, D. A., Corcoran, S. L., Yates, J. W., Pearce, N. J., Rausch, O. L., Murphy, G. J., Carter, P. S., Roxbee Cox, L. et al. (2000). Selective small molecule inhibitors of glycogen synthase kinase-3 modulate glycogen metabolism and gene transcription. *Chem Biol.* 7, 793-803.
- Cohen, P., Alessi, D. R. and Cross, D. A. (1997). PDK1, one of the missing links in insulin signal transduction? *FEBS Lett.* 410, 3-10.
- Cross, D. A., Alessi, D. R., Cohen, P., Andjelkovich, M. and Hemmings, B. A. (1995). Inhibition of glycogen synthase kinase-3 by insulin mediated by protein kinase B. *Nature* 378, 785-789.
- Cross, D. A., Culbert, A. A., Chalmers, K. A., Facci, L., Skaper, S. D. and Reith, A. D. (2001). Selective small-molecule inhibitors of glycogen synthase kinase-3 activity protect primary neurones from death. *J Neurochem.* 77, 94-102.
- Dajani, R., Fraser, E., Roe, S. M., Young, N., Good, V., Dale, T. C. and Pearl, L. H. (2001). Crystal structure of glycogen synthase kinase 3 beta: structural basis for phosphate-primed substrate specificity and autoinhibition. *Cell* 105, 721-732.
- Damiens, E., Baratte, B., Marie, D., Eisenbrand, G. and Meijer, L. (2001). Anti-mitotic properties of indirubin-3'-monoxime, a CDK/GSK-3 inhibitor: induction of endoreplication following prophase arrest. *Oncogene* 20, 3786-37897.
- De Ferrari, G. V. and Inestrosa, N. C. (2000). Wnt signaling function in Alzheimer's disease. *Brain Res Rev* 33, 1-12.
- De Sarno, P., Li, X. and Jope, R. S. (2002). Regulation of Akt and glycogen synthase kinase-3beta phosphorylation by sodium valproate and lithium. *Neuropharmacology* 43, 1158-1164.
- Dent, P., Campbell, D. G., Hubbard, M. J. and Cohen, P. (1989). Multisite phosphorylation of the glycogen-binding subunit of protein phosphatase-1G by cyclic AMP-dependent protein kinase and glycogen synthase kinase-3. *FEBS Lett.* 248, 67-72.
- Dent, P., Lavoinne, A., Nakielny, S., Caudwell, F. B., Watt, P. and Cohen, P. (1990). The molecular mechanism by which insulin stimulates glycogen synthesis in mammalian skeletal muscle. *Nature* 348, 302-308.
- Detera-Wadleigh, S. D. (2001). Lithium-related genetics of bipolar disorder. *Ann Med* 33, 272-285.
- Diehl, J. A., Cheng, M., Roussel, M. R. and Sherr, C. J. (1998). Glycogen synthase kinase-3beta regulates cyclin D1 proteolysis and subcellular localization. *Genes Dev.* 12, 3499-3511.
- Dierick, H. and Bejovert, A. (1999). Cellular mechanisms of wingless/Wnt signal transduction. *Curr Top Dev Biol.* 43, 153-190.
- Ding, V. W., Chen, R. H. and McCormick, F. (2000). Differential regulation of glycogen synthase kinase 3beta by insulin and Wnt signaling. *J Biol Chem.* 275, 32475-32481.
- Eldar-Finkelman, H. (2002). Glycogen synthase kinase 3: an emerging therapeutic target. *Trends Mol Med.* 8, 126-132.
- Eldar-Finkelman, H. and Krebs, E. G. (1997). Phosphorylation of insulin receptor substrate 1 by glycogen synthase kinase 3 impairs insulin action. *Proc Natl Acad Sci USA* 94, 9660-9664.
- Embi, N., Ryfatt, D. B. and Cohen, P. (1980). Glycogen synthase kinase-3 from rabbit skeletal muscle. Separation from cyclic-AMP-dependent protein kinase and phosphorylase kinase. *Eur J Biochem.* 107, 519-527.
- Fang, X., Yu, S., Tanyi, J. L., Lu, Y., Woodgett, J. R. and Mills, G. B. (2002). Convergence of multiple signaling cascades at glycogen synthase kinase 3: Edge receptor-mediated phosphorylation and inactivation by lysophosphatidic acid through a protein kinase C-dependent intracellular pathway. *Mol Cell Biol.* 22, 2099-2110.
- Fang, X., Yu, S. X., Lu, Y., Bast, R. C., Jr., Woodgett, J. R. and Mills, G. B. (2000). Phosphorylation and inactivation of glycogen synthase kinase 3 by protein kinase A. *Proc Natl Acad Sci USA* 97, 11960-11965.
- Farr, G. H., 3rd, Ferkey, D. M., Yost, C., Pierce, S. B., Weaver, C. and Kimelman, D. (2000). Interaction among GSK-3, GBP, axin, and APC in *Xenopus* axis specification. *J Cell Biol.* 148, 691-702.
- Ferkey, D. M. and Kimelman, D. (2002). Glycogen synthase kinase-3 beta mutagenesis identifies a common binding domain for GBP and Axin. *J Biol Chem.* 277, 16147-16152.
- Fiol, C. J., Mahrenholz, A. M., Wang, Y., Roeske, R. W. and Roach, P. J. (1987). Formation of protein kinase recognition sites by covalent modification of the substrate. Molecular mechanism for the synergistic action of casein kinase II and glycogen synthase kinase 3. *J Biol Chem.* 262, 14042-14048.
- Fiol, C. J., Haseman, J. H., Wang, Y. H., Roach, P. J., Roeske, R. W., Kowalczyk, M. and DePaoli-Roach, A. A. (1988). Phosphoserine as a recognition determinant for glycogen synthase kinase-3: phosphorylation of a synthetic peptide based on the G-component of protein phosphatase-1. *Arch Biochem Biophys.* 267, 797-802.
- Fiol, C. J., Wang, A., Roeske, R. W. and Roach, P. J. (1990). Ordered multisite protein phosphorylation. Analysis of glycogen synthase kinase 3 action using model peptide substrates. *J Biol Chem.* 265, 6061-6065.
- Fiol, C. J., Williams, J. S., Chou, C. H., Wang, Q. M., Roach, P. J. and Andrisani, O. M. (1994). A secondary phosphorylation of CREB341 at Ser129 is required for the cAMP-mediated control of gene expression. A role for glycogen synthase kinase-3 in the control of gene expression. *J Biol Chem.* 269, 32187-32193.
- Forlenza, O. V., Spink, J. M., Dayanandan, R., Anderton, B. H., Olesen, O. F. and Lovestone, S. (2000). Muscarinic agonists reduce tau phosphorylation in non-neuronal cells via GSK-3beta inhibition and in neurons. *J Neural Transm.* 107, 1201-1212.
- Frame, S. and Cohen, P. (2001). GSK3 takes centre stage more than 20 years after its discovery. *Biochem J.* 359, 1-16.
- Frame, S., Cohen, P. and Biondi, R. M. (2001). A common phosphate binding site explains the unique substrate specificity of GSK3 and its inactivation by phosphorylation. *Mol Cell* 7, 1321-1327.
- Franca-Koh, J., Yeo, M., Fraser, E., Young, N. and Dale, T. C. (2002). The regulation of glycogen synthase kinase-3 nuclear export by Frat/GBP. *J Biol Chem.* 277, 43844-43848.
- Fraser, E., Young, N., Dajani, R., Franca-Koh, J., Ryves, J., Williams, R. S., Yeo, M., Webster, M. T., Richardson, C., Smalley, M. J. et al. (2002). Identification of the Axin and Frat binding region of glycogen synthase kinase-3. *J Biol Chem.* 277, 2176-2185.
- Freemantle, S. J., Portland, H. B., Ewings, K., Dmitrovsky, E., DiPetrillo, K., Spinella, M. J. and Dmitrovsky, E. (2002). Characterization and tissue-

- specific expression of human GSK-3-binding proteins FRAT1 and FRAT2. *Gene* 291, 17-27.
- Gao, Z. H., Seeling, J. M., Hill, V., Yochum, A. and Virshup, D. M. (2002). Casein kinase I phosphorylates and destabilizes the beta-catenin degradation complex. *Proc. Natl. Acad. Sci. USA* 99, 1182-1187.
- Ghosh, S. and Karin, M. (2002). Missing pieces in the NF-kappaB puzzle. *Cell* 109, S81-96.
- Gould, T. D. and Manji, H. K. (2002). The Wnt signaling pathway in bipolar disorder. *Neuroscientist* 8, 497-511.
- Grimes, C. A. and Jope, R. S. (2001). The multifaceted roles of glycogen synthase kinase 3beta in cellular signaling. *Prog. Neurobiol.* 65, 391-426.
- Hagen, T. and Vidal-Puig, A. (2002). Characterization of the phosphorylation of beta-catenin at the GSK-3 priming site Ser45. *Biochem. Biophys. Res. Commun.* 294, 324-328.
- Hagen, T., di Daniel, E., Culbert, A. A. and Reith, A. D. (2002). Expression and characterization of GSK-3 mutants and their effect on beta-catenin phosphorylation in intact cells. *J. Biol. Chem.* 277, 23330-23335.
- Hall, A. C., Brennan, A., Goold, R. G., Cleverley, K., Lucas, F. R., Gordon-Weeks, P. R. and Salinas, P. C. (2002). Valproate regulates GSK-3-mediated axonal remodeling and synapsin 1 clustering in developing neurons. *Mol. Cell. Neurosci.* 20, 257-270.
- Hanger, D. P., Hughes, K., Wooggett, J. R., Brion, J. P. and Anderton, B. H. (1992). Glycogen synthase kinase-3 induces Alzheimer's disease-like phosphorylation of tau: generation of paired helical filament epitopes and neuronal localisation of the kinase. *Neurosci Lett.* 147, 58-62.
- Hartigan, J. A. and Johnson, G. V. (1999). Transient increases in intracellular calcium result in prolonged site-selective increases in Tau phosphorylation through a glycogen synthase kinase 3beta-dependent pathway. *J. Biol. Chem.* 274, 21395-21401.
- Hartigan, J. A., Xiong, W. C. and Johnson, G. V. (2001). Glycogen synthase kinase 3beta is tyrosine phosphorylated by PYK2. *Biochem. Biophys. Res. Commun.* 284, 485-489.
- Hers, I., Tavare, J. M. and Denton, R. M. (1999). The protein kinase C inhibitors bisindolylmaleimide I (GF 109203x) and IX (Ro 31-8220) are potent inhibitors of glycogen synthase kinase-3 activity. *FEBS Lett.* 460, 433-436.
- Hinoi, T., Yamamoto, H., Kishida, M., Takada, S., Kishida, S. and Kikuchi, A. (2000). Complex formation of adenomatous polyposis coli gene product and axin facilitates glycogen synthase kinase-3 beta-dependent phosphorylation of beta-catenin and down-regulates beta-catenin. *J. Biol. Chem.* 275, 34399-34406.
- Hoeflich, K. P., Luo, J., Ruble, E. A., Tsao, M. S., Jin, O. and Woodgett, J. R. (2000). Requirement for glycogen synthase kinase-3beta in cell survival and NF-kappaB activation. *Nature* 406, 86-90.
- Huelsken, J. and Behrens, J. (2002). The Wnt signalling pathway. *J. Cell Sci.* 115, 3977-3978.
- Hughes, K., Ramakrishna, S., Benjamin, W. B. and Woodgett, J. R. (1992). Identification of multifunctional ATP-citrate lyase kinase as the alpha-isozyme of glycogen synthase kinase-3. *Biochem. J.* 288, 309-314.
- Hughes, K., Nikolakaki, E., Plyte, S. E., Totty, N. F. and Woodgett, J. R. (1993). Modulation of the glycogen synthase kinase-3 family by tyrosine phosphorylation. *EMBO J.* 12, 803-808.
- Ikeda, S., Kishida, S., Yamamoto, H., Murali, H., Koyama, S. and Kikuchi, A. (1998). Axin, a negative regulator of the Wnt signaling pathway, forms a complex with GSK-3beta and beta-catenin and promotes GSK-3beta-dependent phosphorylation of beta-catenin. *EMBO J.* 17, 1371-1384.
- Iouz, R., Kaidanovich, O., Gurwitz, D. and Eldar-Finkelman, H. (2002). Inhibition of glycogen synthase kinase-3beta by bivalent zinc ions: insight into the insulin-mimetic action of zinc. *Biochem. Biophys. Res. Commun.* 295, 102-106.
- Ingham, P. W. and McMahon, A. P. (2001). Hedgehog signaling in animal development: paradigms and principles. *Genes Dev.* 15, 3059-3087.
- Jang, M. K., Goo, Y. H., Sohn, Y. C., Kim, Y. S., Lee, S. K., Kang, H., Cheong, J. and Lee, J. W. (2001). Ca²⁺/calmodulin-dependent protein kinase IV stimulates nuclear factor-kappa B transactivation via phosphorylation of the p65 subunit. *J. Biol. Chem.* 276, 20005-20010.
- Jho, E., Lomvardas, S. and Costantini, F. (1999). A GSK3beta phosphorylation site in axin modulates interaction with beta-catenin and Tcf-mediated gene expression. *Biochem. Biophys. Res. Commun.* 266, 28-35.
- Jia, J., Amanai, K., Wang, G., Tang, J., Wang, B. and Jiang, J. (2002). Shaggy/GSK3 antagonizes Hedgehog signalling by regulating Cubitus interruptus. *Nature* 416, 548-552.
- Johnson, R. L., Rothman, A. L., Xie, J., Goodrich, L. V., Bare, J. W., Bonifas, J. M., Quinn, A. G., Myers, R. M., Cox, D. R., Epstein, E. H., Jr et al. (1996). Human homolog of patched, a candidate gene for the basal cell nevus syndrome. *Science* 272, 1668-1671.
- Kaidanovich, O. and Eldar-Finkelman, H. (2002). The role of glycogen synthase kinase-3 in insulin resistance and Type 2 diabetes. *Expert Opin. Ther. Targets* 6, 555-561.
- Khaled, M., Larribere, L., Bille, K., Aberdam, E., Ortonne, J. P., Ballotti, R. and Bertolotto, C. (2002). Glycogen synthase kinase 3b is activated by cAMP and plays an active role in the regulation of melanogenesis. *J. Biol. Chem.* 277, 33690-33697.
- Kim, L., Harwood, A. and Kimmel, A. R. (2002). Receptor-dependent and tyrosine phosphatase-mediated inhibition of GSK3 regulates cell fate choice. *Dev. Cell* 3, 523-532.
- Kim, L., Liu, J. and Kimmel, A. R. (1999). The novel tyrosine kinase ZAK1 activates GSK3 to direct cell fate specification. *Cell* 99, 399-408.
- Kirschenbaum, F., Hsu, S. C., Cordell, B. and McCarthy, J. V. (2001). Glycogen synthase kinase-3beta regulates presenilin 1 C-terminal fragment levels. *J. Biol. Chem.* 276, 30701-30707.
- Kishida, M., Hino, S., Michie, T., Yamamoto, H., Kishida, S., Fukui, A., Asashima, M. and Kikuchi, A. (2001). Synergistic activation of the Wnt signaling pathway by Dvl and casein kinase epsilon. *J. Biol. Chem.* 276, 33147-33155.
- Klein, P. S. and Melton, D. A. (1996). A molecular mechanism for the effect of lithium on development. *Proc. Natl. Acad. Sci. USA* 93, 8455-8459.
- Knockaert, M., Wiekking, K., Schmitt, S., Leost, M., Grant, K. M., Mottram, J. C., Kunick, C. and Meijer, L. (2002). Intracellular targets of Paulownes. Identification following affinity purification on immobilized inhibitor. *J. Biol. Chem.* 277, 25493-25501.
- Krause, U., Bertrand, L., Maisin, L., Rosa, M. and Hue, L. (2002). Signalling pathways and combinatory effects of insulin and amino acids in isolated rat hepatocytes. *Eur. J. Biochem.* 269, 3742-3750.
- Leclerc, S., Garnier, M., Hoessl, R., Marko, D., Bibb, J. A., Snyder, G. L., Greengard, P., Biernat, J., Wu, Y. Z., Mandelkow, E. M. et al. (2001). Indirubins inhibit glycogen synthase kinase-3 beta and CDK5/p25, two protein kinases involved in abnormal tau phosphorylation in Alzheimer's disease. A property common to most cyclin-dependent kinase inhibitors? *J. Biol. Chem.* 276, 251-260.
- Lee, E., Salic, A. and Kirschner, M. W. (2001). Physiological regulation of [beta]-catenin stability by Tcf3 and CK1epsilon. *J. Cell Biol.* 154, 983-993.
- Leost, M., Schultz, C., Link, A., Wu, Y. Z., Biernat, J., Mandelkow, E. M., Bibb, J. A., Snyder, G. L., Greengard, P., Zaharevitz, D. W. et al. (2000). Paulownes are potent inhibitors of glycogen synthase kinase-3beta and cyclin-dependent kinase 5/p25. *Eur. J. Biochem.* 267, 5983-5994.
- Lesort, M., Jope, R. S. and Johnson, G. V. (1999). Insulin transiently increases tau phosphorylation: involvement of glycogen synthase kinase-3beta and Fyn tyrosine kinase. *J. Neurochem.* 72, 576-584.
- Li, M., Wang, X., Melzner, M. K., Laessig, T., Birnbaum, M. J. and Heidereich, K. A. (2000). Cyclic AMP promotes neuronal survival by phosphorylation of glycogen synthase kinase 3beta. *Mol. Cell. Biol.* 20, 9356-9363.
- Li, Q., van Antwerp, D., Mercurio, F., Lee, K. F. and Verma, I. M. (1999). Severe liver degeneration in mice lacking the IkappaB kinase 2 gene. *Science* 284, 321-325.
- Li, X., Bijur, G. N. and Jope, R. S. (2002). Glycogen synthase kinase-3beta, mood stabilizers, and neuroprotection. *Bipolar Disord.* 4, 137-144.
- Li, Y., Bharati, A., Chen, D., Gong, J. and Kufe, D. (1998). Interaction of glycogen synthase kinase 3beta with the DP3/MUC1 carcinoma-associated antigen and beta-catenin. *Mol. Cell. Biol.* 18, 7216-7224.
- Liu, C., Li, Y., Semenov, M., Han, C., Baeg, G. H., Tan, Y., Zhang, Z., Lin, X. and He, X. (2002). Control of beta-catenin phosphorylation/degradation by a dual-kinase mechanism. *Cell* 108, 837-847.
- Lochhead, P. A., Coghlan, M., Rice, S. Q. and Sutherland, C. (2001). Inhibition of GSK-3 selectively reduces glucose-6-phosphatase and phosphatase and phosphoenolpyruvate carboxykinase gene expression. *Diabetes* 50, 937-946.
- Maccioni, R. B., Munoz, J. P. and Barbelot, L. (2001). The molecular bases of Alzheimer's disease and other neurodegenerative disorders. *Arch. Med. Res.* 32, 367-381.
- Manji, H. K., Moore, G. J. and Chen, G. (1999). Lithium at 50: have the neuroprotective effects of this unique cation been overlooked? *Biol. Psychiatry* 46, 929-940.

- Manoukian, A. S. and Woodgett, J. R. (2002). Role of glycogen synthase kinase-3 in cancer: regulation by Wnts and other signaling pathways. *Adv. Cancer Res.* 84, 203-229.
- Martinez, S., Inonog, S., Manoukian, A. S. and Young, M. W. (2001). A role for the segment polarity gene shaggy/GSK-3 in the *Drosophila* circadian clock. *Cell* 105, 769-779.
- Martinez, A., Alonso, M., Castro, A., Perez, C. and Moreno, F. J. (2002a). First non-ATP competitive glycogen synthase kinase 3 beta (GSK-3beta) inhibitors: thiadiazolidinones (TDZD) as potential drugs for the treatment of Alzheimer's disease. *J. Med. Chem.* 45, 1292-1299.
- Martinez, A., Castro, A., Dorronsoro, I. and Alonso, M. (2002b). Glycogen synthase kinase 3 (GSK-3) inhibitors as new promising drugs for diabetes, neurodegeneration, cancer, and inflammation. *Med. Res. Rev.* 22, 373-384.
- Mattson, M. P. (2001). Neuronal death and GSK-3beta: a tau fetish? *Trends Neurosci.* 24, 255-256.
- McKay, R. M., Peters, J. M. and Graff, J. M. (2001). The casein kinase 1 family in Wnt signaling. *Dev. Biol.* 235, 388-396.
- Meijer, L., Thunnissen, A. M., White, A. W., Garnier, M., Nikolic, M., Tsai, L. H., Walter, J., Cleverley, K. E., Sallinas, P. C., Wu, Y. Z. et al. (2000). Inhibition of cyclin-dependent kinases, GSK-3beta and CK1 by hymenialdisine, a marine sponge constituent. *Chem. Biol.* 7, 51-63.
- Mettey, Y., Gompel, M., Thomas, V., Garnier, M., Leost, M., Ceballos-Picó, J., Noble, M., Endicott, J., Vierfond, J. M. and Meijer, L. (2003). Aloisines, a new family of CDK/GSK-3 inhibitors. SAR study, crystal structure in complex with CDK2, enzyme selectivity, and cellular effects. *J. Med. Chem.* 46, 222-236.
- Miller, J. R. (2002). The Wnts. *Genome Biol.* 3, REVIEWS3001.
- Morfini, G., Szabaday, G., Elluru, R., Ratner, N. and Brady, S. T. (2002). Glycogen synthase kinase 3 phosphorylates kinesin light chains and negatively regulates kinesin-based motility. *EMBO J.* 21, 281-293.
- Mukai, K., Ishiguro, K., Sano, Y. and Fujita, S. C. (2002). Alternative splicing isoform of tau protein kinase I/glycogen synthase kinase 3beta. *J. Neurochem.* 81, 1073-1083.
- Murai, H., Okazaki, M. and Kikuchi, A. (1996). Tyrosine dephosphorylation of glycogen synthase kinase-3 is involved in its extracellular signal-dependent inactivation. *FEBS Lett.* 392, 153-160.
- Novak, A. and Dedhar, S. (1999). Signaling through beta-catenin and Lef/Tcf. *Cell Mol. Life Sci.* 56, 523-537.
- Palacino, J. J., Murphy, M. P., Murayama, O., Iwasaki, K., Fujiwara, M., Takashima, A., Golde, T. E. and Wolozin, B. (2001). Presenilin 1 regulates beta-catenin-mediated transcription in a glycogen synthase kinase-3-independent fashion. *J. Biol. Chem.* 276, 38563-38569.
- Park, I. K., Roach, P., Bondor, J., Fox, S. P. and DePaoli-Roach, A. A. (1994). Molecular mechanism of the synergistic phosphorylation of phosphatase inhibitor-2. Cloning, expression, and site-directed mutagenesis of inhibitor-2. *J. Biol. Chem.* 269, 944-954.
- Plyta, S. E., O'Donovan, E., Woodgett, J. R. and Harwood, A. J. (1999). Glycogen synthase kinase-3 (GSK-3) is regulated during *Dictyostelium* development via the serpentine receptor cAR3. *Development* 126, 325-333.
- Polakis, P. (1997). The adenomatous polyposis coli (APC) tumor suppressor. *Biochim. Biophys. Acta* 1332, F127-F147.
- Polakis, P. (2000). Wnt signaling and cancer. *Genes Dev.* 14, 1837-1851.
- Polakis, P. (2002). Casein Kinase 1: A Wnt'er of disconnect. *Curr. Biol.* 12, R499.
- Price, M. A. and Kalderon, D. (2002). Proteolysis of the Hedgehog signaling effector Cubitus interruptus requires phosphorylation by Glycogen Synthase Kinase 3 and Casein Kinase 1. *Cell* 108, 823-835.
- Pulverer, B. J., Fisher, C., Vousden, K., Littlewood, T., Evan, G. and Woodgett, J. R. (1994). Site-specific modulation of c-Myc cotransformation by residues phosphorylated in vivo. *Oncogene* 9, 59-70.
- Rothwarf, D. M. and Karin, M. (1999). The NF-kappa B activation pathway: a paradigm in information transfer from membrane to nucleus. *Sci. STKE* 1999, RE1.
- Rubinfeld, B., Albert, I., Porfiri, E., Fiol, C., Munemitsu, S. and Polakis, P. (1996). Binding of GSK3beta to the APC-beta-catenin complex and regulation of complex assembly. *Science* 272, 1023-1026.
- Ruiz i Altaba, A. (1999) Gli proteins and Hedgehog signaling: development and cancer. *Trends Genet.* 15, 418-425.
- Ryves, W. J. and Harwood, A. J. (2001). Lithium inhibits glycogen synthase kinase-3 by competition for magnesium. *Biochem. Biophys. Res. Commun.* 280, 720-725.
- Ryves, W. J., Dajani, R., Pearl, L. and Harwood, A. J. (2002). Glycogen synthase kinase-3 inhibition by lithium and beryllium suggests the presence of two magnesium binding sites. *Biochem. Biophys. Res. Commun.* 290, 967-972.
- Saito, Y., Vandeneheede, J. R. and Cohen, P. (1994). The mechanism by which epidermal growth factor inhibits glycogen synthase kinase 3 in A431 cells. *Biochem. J.* 303, 27-31.
- Sakanaka, C. (2002). Phosphorylation and regulation of beta-catenin by casein kinase 1 epsilon. *J. Biochem. (Tokyo)* 132, 697-703.
- Sayas, C. L., Avila, J. and Wandosell, F. (2002). Glycogen synthase kinase-3 is activated in neuronal cells by galphai 2 and galphai 3 by rho-independent and rho-dependent mechanisms. *J. Neurosci.* 22, 6863-6875.
- Schwabe, R. F. and Brenner, D. A. (2002). Role of glycogen synthase kinase-3 in TNF-alpha-induced NF-kappaB activation and apoptosis in hepatocytes. *Am. J. Physiol. Gastrointest. Liver Physiol.* 283, G204-G211.
- Schwarz-Romond, T., Asbrand, C., Bakkers, J., Kuhl, M., Schaeffer, H. J., Huelsken, J., Behrens, J., Hammerschmidt, M. and Birchmeier, W. (2002). The ankyrin repeat protein Diversin recruits Casein kinase epsilon to the beta-catenin degradation complex and acts in both canonical Wnt and Wnt/JNK signaling. *Genes Dev.* 16, 2073-2084.
- Sears, R., Nuekolls, F., Haura, E., Taye, Y., Tamai, K. and Nevins, J. R. (2000). Multiple Ras-dependent phosphorylation pathways regulate Myc protein stability. *Genes Dev.* 14, 2501-2514.
- Seidensticker, M. J. and Behrens, J. (2000). Biochemical interactions in the wnt pathway. *Biochim. Biophys. Acta* 1495, 168-182.
- Sharpe, C., Lawrence, N. and Martinez Arias, A. (2001). Wnt signalling: a theme with nuclear variations. *Bioessays* 23, 311-318.
- Siegfried, E. and Perrimon, N. (1994). *Drosophila* wingless: a paradigm for the function and mechanism of Wnt signalling. *Bioessays* 16, 395-404.
- Siegfried, E., Perkins, L. A., Capati, T. M. and Perrimon, N. (1990). Putative protein kinase product of the *Drosophila* segment-polarity gene zeste-white3. *Nature* 345, 825-829.
- Slingsby, L. P., Denslow, N. D. and Wahba, A. J. (1996). Modulation of rabbit reticulocyte guanine nucleotide exchange factor activity by casein kinases 1 and 2 and glycogen synthase kinase 3. *Biochemistry* 35, 3206-3212.
- Smalley, M. J. and Dale, T. C. (1999). Wnt signalling in mammalian development and cancer. *Cancer Metastasis Rev.* 18, 215-230.
- Smith, D. G., Buffet, M., Fenwick, A. E., Haigh, D., Ife, R. J., Saunders, M., Slingsby, B. P., Stacey, R. and Ward, R. W. (2001). 3-Anilino-4-arylmaleimides: potent and selective inhibitors of glycogen synthase kinase-3 (GSK-3). *Bioorg. Med. Chem. Lett.* 11, 635-639.
- Stambolic, V., Ruel, L. and Woodgett, J. R. (1996). Lithium inhibits glycogen synthase kinase-3 activity and mimics wingless signalling in intact cells. *Curr. Biol.* 6, 1664-1668.
- Steiner, M. R., Holtsberg, F. W., Keller, J. N., Mattson, M. P. and Steiner, S. M. (2000). Lysophosphatidic acid induction of neuronal apoptosis and necrosis. *Ann. N.Y Acad. Sci.* 905, 132-141.
- Sumoy, L., Kiefer, J. and Kimelman, D. (1999). Conservation of intracellular Wnt signalling components in dorsal-ventral axis formation in zebrafish. *Dev. Genes Evol.* 209, 48-58.
- Taijale, J. and Beachy, P. A. (2001). The Hedgehog and Wnt signalling pathways in cancer. *Nature* 411, 349-354.
- Takeda, K., Takemoto, C., Kobayashi, I., Watanabe, A., Nobukuni, Y., Fisher, D. E. and Tachibana, M. (2000). Ser298 of MITF, a mutation site in Waardenburg syndrome type 2, is a phosphorylation site with functional significance. *Hum. Mol. Genet.* 9, 125-132.
- Tanji, C., Yamamoto, H., Yorioka, N., Kohno, N., Kikuchi, K. and Kikuchi, A. (2002). A-kinase anchoring protein AKAP220 binds to glycogen synthase kinase-3beta (GSK-3beta) and mediates protein kinase A-dependent inhibition of GSK-3beta. *J. Biol. Chem.* 277, 36955-36961.
- Ter Haar, E., Coll, J. T., Austen, D. A., Hsiao, H. M., Swenson, L. and Jain, J. (2001). Structure of GSK3beta reveals a primed phosphorylation mechanism. *Nat. Struct. Biol.* 8, 593-596.
- Terruzzi, I., Allibardi, S., Bendinelli, P., Maroni, P., Piccoletti, R., Vesco, F., Samaja, M. and Luzzi, L. (2002). Amino acid- and lipid-induced insulin resistance in rat heart: molecular mechanisms. *Mol. Cell. Endocrinol.* 190, 135-145.
- Thomas, G. M., Frame, S., Goedert, M., Nathke, I., Polakis, P. and Cohen, P. (1999). A GSK3-binding peptide from FRAT1 selectively inhibits the GSK3-catalysed phosphorylation of axin and beta-catenin. *FEBS Lett.* 458, 247-251.
- van Noort, M., Meeldijk, J., van der Zee, R., Destree, O. and Clevers, H. (2002). Wnt signalling controls the phosphorylation status of beta-catenin. *J. Biol. Chem.* 277, 17901-17905.
- Wang, Q. M., Fiol, C. J., DePaoli-Roach, A. A. and Roach, P. J. (1994). Glycogen synthase kinase-3 beta is a dual specificity kinase differentially

- regulated by tyrosine and serine/threonine phosphorylation. *J. Biol. Chem.* 269, 14566-14574.
- Wang, D., Westerheide, S. D., Hanson, J. L. and Baldwin, A. S., Jr (2000). Tumor necrosis factor alpha-induced phosphorylation of RelA/p65 on Ser529 is controlled by casein kinase II. *J. Biol. Chem.* 275, 32592-32597.
- Watcharasit, P., Bijur, G. N., Zmijewski, J. W., Song, L., Zmijewska, A., Chen, X., Johnson, G. V. and Jope, R. S. (2002). Direct, activating interaction between glycogen synthase kinase-3beta and p53 after DNA damage. *Proc. Natl. Acad. Sci. USA* 99, 7951-7955.
- Welsh, G. I. and Proud, C. G. (1993). Glycogen synthase kinase-3 is rapidly inactivated in response to insulin and phosphorylates eukaryotic initiation factor eIF-2B. *Biochem. J.* 294, 625-629.
- Williams, R. S., Cheng, L., Mudge, A. W. and Harwood, A. J. (2002). A common mechanism of action for three mood-stabilizing drugs. *Nature* 417, 292-295.
- Woodgett, J. R. (1990). Molecular cloning and expression of glycogen synthase kinase-3/factor A. *EMBO J.* 9, 2431-2438.
- Woodgett, J. R. (2001). Judging a protein by more than its name: GSK-3. *Sci STKE* 2001, RE12.
- Woodgett, J. R. and Cohen, P. (1984). Multisite phosphorylation of glycogen synthase. Molecular basis for the substrate specificity of glycogen synthase kinase-3 and casein kinase-II (glycogen synthase kinase-5). *Biochim. Biophys. Acta* 788, 339-347.
- Yamamoto, H., Kishida, S., Kishida, M., Ikeda, S., Takada, S. and Kikuchi, A. (1999). Phosphorylation of axin, a Wnt signal negative regulator, by glycogen synthase kinase-3beta regulates its stability. *J. Biol. Chem.* 274, 10681-10684.
- Yanagawa, S., Matsuda, Y., Lee, J. S., Matsubayashi, H., Sese, S., Kadokawa, T. and Ishimoto, A. (2002). Casein kinase I phosphorylates the Armadillo protein and induces its degradation in *Drosophila*. *EMBO J.* 21, 1733-1742.
- Yost, C., Farr, G. H., 3rd, Pierce, S. B., Ferkey, D. M., Chen, M. M. and Kimelman, D. (1998). GBP, an inhibitor of GSK-3, is implicated in Xenopus development and oncogenesis. *Cell* 93, 1031-1041.
- Yost, C., Torres, M., Miller, J. R., Huang, E., Kimelman, D. and Moon, R. T. (1996). The axis-inducing activity, stability, and subcellular distribution of beta-catenin is regulated in *Xenopus* embryos by glycogen synthase kinase 3. *Genes Dev.* 10, 1443-1454.
- Yuan, H., Mao, J., Li, L. and Wu, D. (1999). Suppression of glycogen synthase kinase activity is not sufficient for leukemia enhancer factor-1 activation. *J. Biol. Chem.* 274, 30419-30423.